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Do self-fertilization and genetic drift promote a very low genetic variability in the allotetraploid *Bulinus truncatus* (Gastropoda: Planorbidae) populations?

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Summary

Bulinus truncatus, one of the intermediate hosts of the genus *Schistosoma* is an hermaphrodite freshwater snail species occupying a variety of environments over almost all Africa. These environments are subjected to large variations in water availability. *B. truncatus* is allotetraploid and its populations exhibit various frequencies of aphallic individuals (unable to reproduce as male). Both traits probably favour a reproduction by self-fertilization. Here we investigate the genetic structure of populations of *B. truncatus* of Niger and Ivory Coast using protein electrophoresis to analyse the influence of the environment and of both the last traits. To obtain an estimate of the true heterozygosity in this allotetraploid species, we analyse independently the two diploid loci at each tetraploid locus. Our study indicates (i) an extremely low intrapopulation polymorphism with most alleles fixed and the total absence of heterozygotes and (ii) low differentiation between populations. These results indicate high gene flow between populations. However, the existence of private alleles sometimes at high frequency, the low polymorphism and the lack of heterozygotes point to the role of both genetic drift and self-fertilization, the second amplifying the genetic consequences of the first.

1. Introduction

Population genetics is basically concerned with the distribution of genetic variability within and among populations, and with the various forces such as genetic drift, gene flow and mating systems modifying this distribution (Slatkin, 1985*a*). The estimation of the relative roles of such forces is crucial in populations of parasite intermediate hosts to understand the transmission of their parasites (Rollinson, 1985). *Bulinus truncatus* is one of the intermediate hosts of the genus *Schistosoma* in Africa (Brown, 1980). A geographic variability of snail susceptibility to schistosomes has been shown (Brown, 1980; Vera *et al.* 1990) requiring a closer analysis of snail population structure. Two aspects of the biology of *B. truncatus* must be pointed out: (i) this species occupies different kinds of more or less temporary habitats (ponds, rivers, irrigation systems, etc.) over various bioclimatic systems (forest, guineo-soudanese savannah, sahelian savannah and saharian area) (Sellin & Boudin, 1981; Betterton, 1984; Doumenge *et al.* 1987; Vera *et al.* 1990). The distribution of populations is patchy and depends on water availability and temperature. Population size therefore fluctuates widely, and genetic drift, extinction and recolonization are important determinants of population genetic structure (Marti & Tanner, 1988; Woolhouse & Chandiwana, 1989; Jarne & Delay, 1991). (ii) *B. truncatus* exhibits two peculiar genetic systems of particular value in the analysis of population genetic structure. The first is aphally which is characterized in hermaphrodite snails by the lack of the copulatory organ, and which prevents outcrossing as male. However, aphallic individuals can outcross as female or self-fertilize (Larambergue, 1939). The determination of aphally is still unclear; some results indicate a genetic determination (Larambergue, 1939) whereas others point strongly to the role of temperature (Schrag & Read, 1992). The frequency of aphallic individuals is highly variable among populations (Larambergue, 1939). With an increasing frequency of aphallics, the

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selfing rate likely increases (Jarne *et al.* 1992*b*). The second peculiar genetic system is tetraploidy which is probably of hybrid origin (Goldman *et al.* 1983). The occurrence of 'fixed heterozygote' phenotypes on protein electrophoresis zymograms has been interpreted in terms of disomic segregation of alleles at homologous loci (Njiokou *et al.* 1993). Tetraploidy could influence the genetic structure of populations because it could select for an increased selfing rate (Lande & Schemske, 1985). Indeed the first event after tetraploidization is likely to be selfing which removes part of the genetic load by eliminating deleterious alleles.

B. truncatus belongs to the *Bulinus tropicus/truncatus* systematic group. Morphological and anatomical characters as defined by Mandahl-Barth (1957) cannot generally distinguish between all species in this group (Brown & Wright, 1972; Brown, 1976). The first protein electrophoretic studies were therefore taxonomic studies using both laboratory stocks and wild individuals of various geographic origins. As a by-product of these studies, a very low intrapopulation polymorphism was shown in *B. truncatus* with slight differences among populations (Jelnes, 1978, 1986; Paggi *et al.* 1978; Wurzinger & Saliba, 1979; Aru *et al.* 1980; Nascetti & Bullini, 1980; Wright & Rollinson, 1981; Mimpfoundi & Greer, 1990*d*). However, no analysis of population genetic structure has been performed in *B. truncatus*, which may be partly due to the unusual difficulty of zymogram interpretation. We rely here on the interpretation of Njiokou *et al.* (1993).

The goal of this study is to analyse the genetic structure of *B. truncatus* populations from Ivory Coast and Niger, and to analyse the influence on this structure of the genetic systems evoked above using samples from a wide range of bioclimatic systems.

2. Materials and methods

(i) *Samples and loci studied*

Seventeen populations of *B. truncatus* from Ivory Coast and Niger (Fig. 1, Table 1) were sampled giving a total of 24 samples, as some populations were sampled two or three times, and 478 individuals. Twenty individuals originating from an Egyptian laboratory stock were used as an electrophoretic control. In some populations, the frequency of aphallic individuals was also estimated by dissecting individuals and checking for the presence of the phallus. The genetic analysis was performed by protein electrophoresis following Jelnes (1979) and Pasteur *et al.* (1988).

Fourteen enzymatic systems have been analysed: isocitrate dehydrogenase (IDH, EC 1.1.1.42); phosphoglucomutase (PGM, EC 2.7.5.1); esterase (EST, EC 3.1.1.1); aspartate-amino-transferase (AAT, EC 2.6.1.1); nucleoside-phosphorylase (NP, EC 2.4.2.1); manose-phosphate-isomerase (MPI, EC 5.3.1.8); xanthine-dehydrogenase (XDH, EC 1.2.1.37); octanol-dehydrogenase (ODH, EC 1.1.1.73); glucose-phosphate-isomerase (GPI, EC 5.3.1.9); hydroxybutyrate-dehydrogenase (HBDH, EC 3.1.1.31); Alpha-glycerophosphate-dehydrogenase (α-GPDH, EC 1.1.1.8); Six-phosphogluconate-dehydrogenase (6-PGD, EC 1.1.1.43); malate-dehydrogenase (MDH, EC 1.1.1.37); peptidase B (PEPB, EC 3.4.1.1). Isozymes were numbered in order of increasing (anodal) mobility for multi-locus systems. The most common allele was used as a standard for relative mobilities.

(ii) *Coding method of electrophoretic data*

B. truncatus is a tetraploid species with a disomic segregation of alleles which means that the hom-

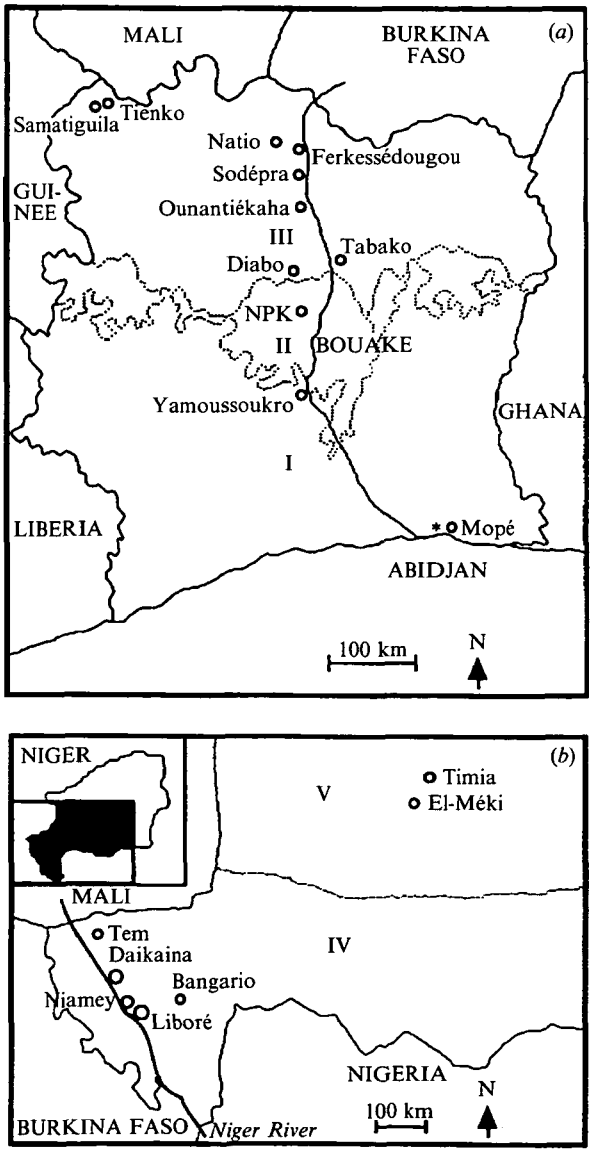

Fig. 1. Sampling sites in Ivory Coast (*a*) and Niger (*b*). I, II, III, IV and V refer to the bioclimatic systems (respectively to forest, preforest area, guineo-soudanese savannah, sahelian savannah and saharian area).

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Table 1. *Country, sampling site, year of sampling, number of individuals per sample (N), mean number of alleles per diploid locus (A), percentage of polymorphic loci (P) and percentage of aphallic individuals (APH) for each population studied*

Country	Site	Year	N	A	P	APH
Ivory Coast	Mopé	1990	6	1·0	3·6	—
	Yamoussoukro	1990	30	1·0	0·0	93
	NPK (a)	1988	25	1·1	7·1	—
	NPK (b)	1989	16	1·0	3·6	61
	Diabo	1989	8	1·0	0·0	60
	Tabako (a)	1988	9	1·0	0·0	—
	Tabako (b)	1989	25	1·0	0·0	100
	Ounantiékaha (a)	1988	30	1·0	3·6	—
	Ounantiékaha (b)	1989	8	1·0	3·6	87
	Ounantiékaha (c)	1990	16	1·0	3·6	86
	Sodepra (a)	1988	29	1·0	0·0	—
	Sodepra (b)	1990	20	1·0	0·0	87
	Ferkessédougou (a)	1988	13	1·0	3·6	—
	Ferkessédougou (b)	1990	30	1·0	3·6	92
	Natio (a)	1988	10	1·0	0·0	—
	Natio (b)	1989	30	1·0	0·0	66
	Tienko	1990	20	1·0	0·0	56
	Samatiguila	1990	15	1·0	0·0	80
Niger	Liboré 9	1991	24	1·0	0·0	—
	Daikaina	1988	28	1·0	0·0	—
	Bangario	1990	37	1·0	0·0	80
	Tem	1990	9	1·0	0·0	—
	El-Meki	1988	22	1·0	0·0	—
	Timia	1988	18	1·1	10·0	—
Egypt		1989	20	1·0	0·0	0
	Mean*		20 (8·75)	1·0 (0·03)	1·7 (2·65)	

* The mean values of N, A and P are given with their standard deviation in parentheses.
— Not estimated.

ologous loci at each diploid set of chromosomes are genetically independent (Allendorf & Thorgaard, 1984). This peculiarity of the genetic system has two important consequences. Firstly, when the two homologous loci are homozygous for different alleles, the tetraploid locus presents two alleles. When all individuals exhibit this pattern within a population, this is referred to as 'fixed heterozygosity'. In this case an individual and its selfed offspring share the same electrophoretic patterns. The zymograms typically show two, three or more bands depending on the quaternary structure of the enzyme studied (Fig. 2). Secondly, when the two homologous loci are homozygous for the same allele or when one of the two loci has been inactivated (functional diploidization *sensu* Ohno, 1970), only one band occurs on the zymograms (Fig. 2). Multi-banded phenotypes are also expected in the case of functional diploidization when the remaining locus bears many alleles. However this case, as well as cases involving two polymorphic diploid loci at the same tetraploid locus, are hardly relevant in *B. truncatus* because of a low polymorphism (see below).

One has then to decide how to analyse the genetic data in the two situations exposed above. Two

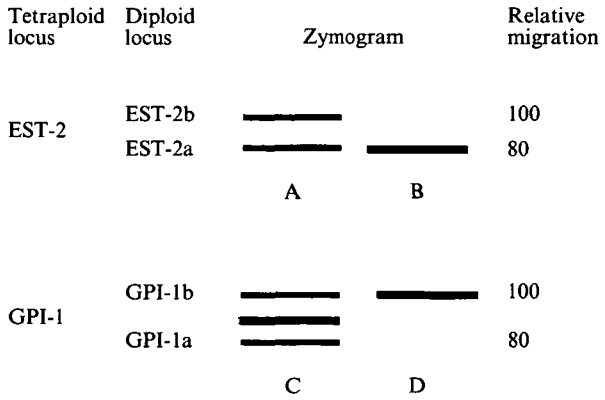

Fig. 2. Coding method for the genetic data on the example of EST-2 (monomeric) and GPI-1 (dimeric) and four individuals A, B, C and D. EST-2 and GPI-1 are tetraploid loci, and the forms 'a' and 'b' represent the homologous diploid loci. Individuals A and C are 'fixed heterozygotes' since their selfed offspring show the same zymogram. These individuals have genotype a-80/80 and b-100/100. Individuals B and D only show a unibanded pattern. These have either two null alleles at one diploid locus and one allele at the other locus, or the same allele at the two loci.

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Table 2. *Enzymes studied with the observed electrophoretic patterns at each tetraploid locus and the alleles identified at each diploid locus*

Enzyme	Tetraploid locus*	Observed electrophoretic patterns	Diploid locus	Identified alleles
EST	EST-2	(80/90, 80/100)	EST-2a	(80, 90, 100, 110)
			EST-2b	(90, 100)
	EST-3	(100/110, 110/110)	EST-3a	(100, 110)
			EST-3b	(100, 110)
PGM	PGM-2	(80/100, 80/105, 100/100)	PGM-2a	(80, 100)
			PGM-2b	(100, 105)
NP	NP-1	(100/100, 100/110, 100/105)	NP-1a	(100)
			NP-1b	(100, 105, 110)
GPI	GPI-1	(80/80, 80/100)	GPI-1a	(80)
			GPI-1b	(80, 100)
IDH	IDH-1	(80/80, 80/100)	IDH-1a	(100)
			IDH-1b	(80, 100)
AAT	AAT-2	(100/100, 100/110)	AAT-2a	(100)
			AAT-2b	(100, 110)
HBDH	HBDH-1	(80/100)	HBDH-1a	(80)
			HBDH-1b	(100)

* The loci EST-1, PGM-1, AAT-1, 6-PGD-1, a-GPD-1, MDH-1, MDH-2, PEPB-1, PEPB-2, ODH-1, MPI-1, XDH-1 and XDH-2 only showed one-banded patterns (100/100) and were considered as monomorphic at each diploid locus.

methods are currently available: (i) the 'fixed heterozygote' coding method for which the variable is the tetraploid locus. For example in Fig. 2, the genotype of individual A at the locus considered is 80/100 and that of individual B is 80/80 or 0/80. With this coding method, the two loci although segregating independently, are considered as a unique locus; (ii) the minimizing coding method (Berrebi *et al.* 1990) for which the two loci (a and b in Fig. 2) are analysed independently. In Fig. 2, individual A has the genotype a-80/80 and b-100/100 whereas individual B is a-80/80 and b-0/0 or b-80/80. In this work, we assumed that a non-functional locus bears the same allele as the functional homologous locus, i.e. that the individual B in Fig. 2 is a-80/80 and b-80/80. Whether null alleles occurred at one of the two functional diploid loci of a given tetraploid locus is not known – no individual had a null allele at both homologous loci of a given tetraploid locus. The consequence of not considering null alleles was to lower the number of alleles per locus and to decrease the value of the genetic distances. The true genetic differentiation of populations is therefore probably higher than that estimated below. An advantage of the second method is to provide a true estimation of heterozygosity which is not possible with the 'fixed heterozygote' coding method.

When only one locus is functional over many populations, one can assume that the other locus bears the same null mutation in all populations which minimizes the interpopulation variability, but does not affect the genetic interpretation. We also assumed that in the few population/locus combinations in which some polymorphism was found (that is with one-banded and multibanded zymogram patterns in the same population), multibanded individuals were homozygous for both diploid loci (a-80/80 and b-100/100 in Fig. 2). We did not assume that these individuals were heterozygous at one of the diploid loci (a-80/100 and b-80/100) since for multibanded patterns at a given locus, we never observed more than two patterns for two alleles in a given population whereas three patterns are expected (Fig. 2). One pattern (for instance, one-banded b-100/100 at EST-2 in Fig. 2) is clearly lacking, which would be unlikely if we assume that the correct interpretation is a-80/100 and b-80/100. On the other hand, assuming a-80/80 and b-100/100 is consistent with the patterns observed. We also independently performed pedigree analyses to test for the disjunction of double- or triple-banded patterns using selfing individuals. No disjunction was observed at the loci studied (these analyses were performed at all polymorphic loci and four populations studied here; see Njiokou *et al.* 1993) which would be unlikely when assuming a-80/100 and b-80/100 patterns.

(iii) *Data analysis*

The alleles and allelic combinations identified are given in Table 2. We estimated the allelic frequencies, the proportion of polymorphic loci per population (P), the allelic diversity (A) and the observed heterozygosity (H_o) (Lewontin, 1974) adopting the minimizing coding method.

When the same population has been sampled over many years, we tested the hypothesis that observed changes in allelic frequencies over time can be attributed solely to sampling error and genetic drift

Table 3. *Allelic frequencies at the polymorphic diploid loci*

Locus...	EST-3b		EST-3a		EST-2b		EST-2a				AAT-2b		IDH-1b		NP-1b			GPI-1b		PGM-2a		PGM-2b	
Alleles...	100	110	100	110	90	100	80	90	100	110	100	110	80	100	100	105	110	80	100	80	100	100	105
Sample*																							
SODa	0·000	1·000	1·000	0·000	0·000	1·000	0·000	1·000	0·000	0·000	1·000	0·000	0·000	1·000	0·000	0·000	1·000	0·000	1·000	0·000	1·000	1·000	0·000
SODb	0·000	1·000	1·000	0·000	0·000	1·000	0·000	1·000	0·000	0·000	1·000	0·000	0·000	1·000	0·000	0·000	1·000	0·000	1·000	0·000	1·000	1·000	0·000
NPKa	0·880	0·120	1·000	0·000	0·000	1·000	0·000	1·000	0·000	0·000	1·000	0·000	0·000	1·000	0·680	0·000	0·320	0·000	1·000	0·000	1·000	1·000	0·000
NPKb	0·875	0·125	1·000	0·000	0·000	1·000	0·000	1·000	0·000	0·000	1·000	0·000	0·000	1·000	1·000	0·000	0·000	0·000	1·000	0·000	1·000	1·000	0·000
OUNa	0·000	1·000	1·000	0·000	0·000	1·000	0·000	1·000	0·000	0·000	1·000	0·000	0·000	1·000	0·733	0·000	0·267	0·000	1·000	0·000	1·000	1·000	0·000
OUNb	0·000	1·000	1·000	0·000	0·000	1·000	0·000	1·000	0·000	0·000	1·000	0·000	0·000	1·000	0·625	0·000	0·375	0·000	1·000	0·000	1·000	1·000	0·000
OUNc	0·000	1·000	1·000	0·000	0·000	1·000	0·000	1·000	0·000	0·000	1·000	0·000	0·000	1·000	0·375	0·000	0·625	0·000	1·000	0·000	1·000	1·000	0·000
FERa	0·000	1·000	1·000	0·000	0·000	1·000	0·000	1·000	0·000	0·000	1·000	0·000	0·000	1·000	0·538	0·000	0·462	0·000	1·000	0·000	1·000	1·000	0·000
FERb	0·000	1·000	1·000	0·000	0·000	1·000	0·000	1·000	0·000	0·000	1·000	0·000	0·000	1·000	0·067	0·000	0·933	0·000	1·000	0·000	1·000	1·000	0·000
NATa	0·000	1·000	1·000	0·000	0·000	1·000	0·000	1·000	0·000	0·000	1·000	0·000	0·000	1·000	0·000	0·000	1·000	0·000	1·000	0·000	1·000	1·000	0·000
NATb	0·000	1·000	1·000	0·000	0·000	1·000	0·000	1·000	0·000	0·000	1·000	0·000	0·000	1·000	0·000	0·000	1·000	0·000	1·000	0·000	1·000	1·000	0·000
TABa	0·000	1·000	1·000	0·000	0·000	1·000	0·000	1·000	0·000	0·000	1·000	0·000	0·000	1·000	1·000	0·000	0·000	0·000	1·000	0·000	1·000	1·000	0·000
TABb	0·000	1·000	1·000	0·000	0·000	1·000	0·000	1·000	0·000	0·000	1·000	0·000	0·000	1·000	1·000	0·000	0·000	0·000	1·000	0·000	1·000	1·000	0·000
DIA	0·000	1·000	1·000	0·000	0·000	1·000	0·000	1·000	0·000	0·000	1·000	0·000	0·000	1·000	1·000	0·000	0·000	0·000	1·000	0·000	1·000	1·000	0·000
MOP	0·000	1·000	1·000	0·000	0·000	1·000	0·833	0·167	0·000	0·000	1·000	0·000	0·000	1·000	1·000	0·000	0·000	0·000	1·000	1·000	0·000	0·000	1·000
TIK	0·000	1·000	1·000	0·000	0·000	1·000	0·000	0·000	0·000	1·000	1·000	0·000	0·000	1·000	1·000	0·000	0·000	0·000	1·000	0·000	1·000	1·000	0·000
YAM	0·000	1·000	1·000	0·000	0·000	1·000	0·000	1·000	0·000	0·000	0·000	1·000	1·000	0·000	1·000	0·000	0·000	0·000	1·000	0·000	1·000	1·000	0·000
SAM	0·000	1·000	1·000	0·000	0·000	1·000	0·000	1·000	0·000	0·000	1·000	0·000	0·000	1·000	1·000	0·000	0·000	0·000	1·000	0·000	1·000	1·000	0·000
DAI	0·000	1·000	0·000	1·000	0·000	1·000	1·000	0·000	0·000	0·000	1·000	0·000	0·000	1·000	0·000	1·000	0·000	0·000	1·000	0·000	1·000	1·000	0·000
ELM	0·000	1·000	1·000	0·000	0·000	1·000	1·000	0·000	0·000	0·000	1·000	0·000	0·000	1·000	1·000	0·000	0·000	0·000	1·000	1·000	0·000	0·000	1·000
BAN	0·000	1·000	1·000	0·000	0·000	1·000	1·000	0·000	0·000	0·000	1·000	0·000	0·000	1·000	1·000	0·000	0·000	1·000	0·000	0·000	1·000	1·000	0·000
TEM	0·000	1·000	1·000	0·000	0·000	1·000	1·000	0·000	0·000	0·000	1·000	0·000	0·000	1·000	1·000	0·000	0·000	0·000	1·000	0·000	1·000	1·000	0·000
TIM	0·000	1·000	1·000	0·000	0·000	1·000	0·611	0·000	0·389	0·000	1·000	0·000	0·000	1·000	1·000	0·000	0·000	0·000	1·000	0·611	0·389	0·000	1·000
LI9	0·000	1·000	1·000	0·000	1·000	0·000	1·000	0·000	0·000	0·000	1·000	0·000	0·000	1·000	1·000	0·000	0·000	0·000	1·000	0·000	1·000	0·000	1·000
EGY	0·000	1·000	1·000	0·000	0·000	1·000	0·000	0·000	0·000	1·000	1·000	0·000	0·000	1·000	1·000	0·000	0·000	0·000	1·000	1·000	0·000	1·000	0·000

* SOD, Sodepra; NPK, Nguessan Pokoukro; OUN, Ounantiékaha; FER, Ferkessédougou; NAT, Natio; TAB, Tabako; DIA, Diabo; MOP, Mopé; TIK, Tienko; YAM, Yamoussoukro; SAM, Samatiguila; DAI, Daikaina; ELM, El-Méki; BAN, Bangario; LI9, Liboré 9; EGY, Egypt.

using the method developed by Waples (1989). Tests were performed using the software TEMPTEST (R. Waples, Version 2.2, 1990). As the actual and effective sizes are unknown in the populations we studied, we performed a battery of tests at each polymorphic locus with N_e (effective size of population) ranging from 50 to 1000, and N (actual size) ranging from N_e to $10N_e$ as suggested by Waples (1989). Tests were performed assuming either 3 or 4 generations per year. Waples' (1989) procedure also assumes discrete generations and samples drawn randomly from the whole population. Our analysis of genetic diversity among populations has been based on two methods. Firstly we estimated Nei's (1978) genetic distance. When more than one sample was available for a population, only one sample was retained to compare populations since the genetic distances among samples were only of the order of 1/1000. The correlation between geographic and genetic distances in Ivory Coast, in Niger and for the whole dataset was tested using a Mantel test (Smouse *et al.* 1986). Levels of significance were calculated on 1000 Monte Carlo samplings using the software GENETIX (F. Bonhomme *et al.*, Version 0.0, 1993). This procedure accounts for the dependence of the entries in each matrix of distance which increases the value of the correlation (Smouse *et al.* 1986). Secondly, we used factorial correspondence analysis (FCA; Benzecri, 1973). This technique is well suited to analyse qualitative data and its adaptation to electrophoretic data is fully described by She *et al.* (1987). The main interest of FCA is the characterization of the genetic information in an n-1 dimensional space, n being the number of alleles. Each axis (dimension) contributes a percentage to the overall variability. The information both within and between populations is not reduced to a single index such as a distance and can be visualized on the most discriminant axes (1, 2 and 3). Moreover the contribution of each allele to each axis is known.

3. Results

(i) *Aphally*

The proportion of aphallic individuals varies from 56 to 100% in Ivory Coast and Niger populations. On the other hand, no aphallic individual was found in the Egyptian laboratory population (Table 1).

Table 4. *Test for the stability of allelic frequencies over generations within the same sampling site at the polymorphic loci. Sample names are as in Table 3*

Sample	Locus	χ^2
NPKa/NPKb	NP-1b	**
	EST-3b	N.S.
OUNa/OUNb/OUNc	NP-1b	*
FERa/FERb	NP-1b	**

* $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; N.S., not significant.

(ii) *Intrapopulation polymorphism*

The allelic frequencies are given in Table 3. At each locus, there is generally one allele either fixed or at a very high frequency. Private alleles (i.e. alleles occurring in only one population) occur in some populations (e.g. EST-2a^{110} in Tienko, Ivory Coast and NP-1b^{105} in Daikaina, Niger) and are generally fixed in those populations. χ^2 values as calculated by Waples's method for the stability of allelic frequencies over generations are given in Table 4. As numerous tests were performed for each locus–population combination, we only give the significance level. Three χ^2 values turned out to differ from zero out of four tests.

The values of A and P show that there is little or no genetic variability in the populations studied. Thirteen out of eighteen populations do not show any polymorphic locus. In the very few polymorphic populations, P varies between 3·4 and 10·3%, i.e. one, two or three loci are polymorphic out of 29 studied. For instance, the major part of the polymorphism in Ivory Coast populations is given by the variation in frequency of NP-1b^{110}. The allelic diversity is never higher than 1.1 and $H_o = 0$ at all loci. No heterozygous genotype was observed at any diploid loci. All polymorphic loci therefore exhibit a large heterozygote deficiency.

(iii) *Genetic diversity among populations*

The genetic distances (Table 5) vary from 0·000 to 0·189 when all populations are included in the analysis. The variation is between 0·000 and 0·177 in Ivory Coast, and 0·031 and 0·148 in Niger. The correlation coefficient between genetic and geographic distances is significantly different from zero using the Mantel test for all populations ($r = 0{\cdot}426$, $P < 0{\cdot}002$) and Ivory Coast populations ($r = 0{\cdot}632$, $P < 0{\cdot}009$) whereas it does not differ from zero among Niger populations ($r = 0{\cdot}314$, $P > 0{\cdot}24$).

The projection of individuals in the first factorial plane (defined by axes 1 and 2) of the FCA is given in Fig. 3. This plane represents 21% of the total information (six axes). Individuals from different populations are often projected within the same area of the plane which indicates a very low polymorphism both within and among populations. However it is possible to distinguish between Ivory Coast and Niger populations along axis 1. Ivory Coast populations are also more similar than are Niger populations. Some populations (Yamoussoukro and Daikaina; Mopé and Timia are separated along axis 2) are clearly separated from the other populations. We did not represent the other dimensions of the space since they do not provide further information. Similarly alleles

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Table 5. *Nei's genetic distances between populations. Sample names are as in Table 3*

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1 SOD	—	0·044	0·019	0·000	0·000	0·035	0·035	0·137	0·071	0·109	0·035	0·190	0·148	0·109	0·071	0·092	0·109	0·109
2 NPK		—	0·027	0·041	0·044	0·031	0·031	0·133	0·067	0·105	0·031	0·132	0·145	0·105	0·067	0·088	0·105	0·105
3 OUN			—	0·015	0·019	0·002	0·002	0·101	0·038	0·074	0·002	0·102	0·113	0·074	0·038	0·057	0·074	0·074
4 FER				—	0·000	0·031	0·031	0·132	0·067	0·104	0·031	0·107	0·144	0·104	0·067	0·087	0·104	0·104
5 NAT					—	0·035	0·035	0·137	0·071	0·109	0·035	0·109	0·148	0·109	0·071	0·092	0·109	0·109
6 TAB						—	0·000	0·098	0·035	0·071	0·000	0·109	0·109	0·071	0·035	0·054	0·071	0·071
7 DIA							—	0·098	0·035	0·071	0·000	0·109	0·109	0·071	0·035	0·054	0·071	0·071
8 MOPE								—	0·104	0·177	0·098	0·150	0·001	0·110	0·072	0·014	0·110	0·066
9 TIE									—	0·109	0·035	0·109	0·109	0·071	0·035	0·054	0·071	0·035
10 YAM										—	0·071	0·189	0·189	0·148	0·109	0·131	0·148	0·148
11 SAM											—	0·109	0·109	0·071	0·035	0·054	0·071	0·071
12 DAI												—	0·148	0·109	0·071	0·107	0·109	0·148
13 ELM													—	0·109	0·071	0·015	0·109	0·071
14 BAN														—	0·035	0·068	0·071	0·109
15 TEM															—	0·031	0·035	0·071
16 TIM																—	0·068	0·046
17 LI9																	—	0·109
18 EGY																		—

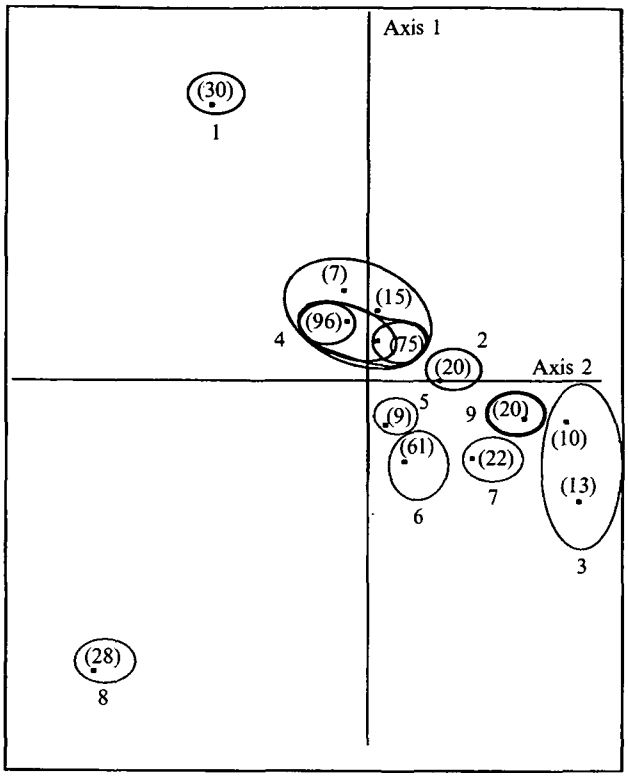

Fig. 3. Projection of individuals in the first plane of the factorial correspondence analysis. The number of individuals projected on the same place is given in parentheses. The numbers outside each ellipsoid refer to the populations projected. Ivory Coast: 1, Yamoussoukro; 2, Tienko; 3, Mopé, 4, other populations. Niger: 3, Timia; 5, Tem; 6, Bangario and Libore; 7, El-Meki; 8, Daikaina. Egypt: 9. Note that individuals from Mopé and Timia are projected on the two points in the area labelled 3.

can be projected in the first plane. This projection indicates which alleles differentiate populations and which alleles do not. For example, Np-1b^{105} separates Daikaina from the other populations (data not shown).

4. Discussion

(i) *Intrapopulation variability*

Our study indicates that the genetic variability as estimated by protein electrophoresis is low in *B. truncatus* over a wide range of bioclimatic systems. This result parallels those obtained by Wright & Rollinson (1981), Jelnes (1986) and Mimpfoundi & Greer (1990*d*). The main cause of this low variability is likely to be genetic drift since tropical freshwater populations regularly experience size restriction and possibly extinction (Brown, 1980; Woolhouse & Chandiwana, 1989; Jarne & Delay, 1991). However it is quite possible that self-fertilization reinforces the action of genetic drift by distributing the different alleles at each locus into different inbred lines. Indeed both aphally and tetraploidy can favour self-fertilization in *B. truncatus* (Jarne *et al.* 1993; Njiokou *et al.* 1993). Although purely aphallic populations seem to be rare, the high frequency of aphallic individuals in the populations studied increases the probability of self-fertilization. A second important result of our study is the absence of heterozygotes at diploid loci, which also points to the role of self-fertilization in *B. truncatus* populations. The third result is the presence of private alleles at high frequencies. Genetic drift is also probably the main candidate to explain how such alleles can be driven to fixation.

In subdivided populations, the loss of genetic variability can be a consequence of selection or genetic drift (Lewontin, 1974). If we assume an island model of population, selection or genetic drift can lead to the fixation of alleles at all loci when there is no gene flow (Slatkin, 1985*a*). However, few examples of the role of selection of the distribution of the molecular genetic variability are available in snails (Selander & Ochman, 1983). Genetic drift is much more likely to have a role in subdivided populations regularly experiencing size restriction or even extinction (Selander, 1976) such as *Bulinus* and *Biomphalaria* populations (Brown, 1980; Marti & Tanner, 1988; Woolhouse & Chandiwana, 1989). Our results on samples from the same population indicate that the distribution of alleles varies over years (see Table 3) which could also indicate the role of genetic drift.

The distribution of the genetic variability within populations is partly due to the mating system (Lewontin, 1974). Self-fertilization increases the homozygosity at all loci compared to cross-fertilization. There is no evidence from electrophoretic studies that *B. truncatus* outcrosses in nature. However, laboratory studies have shown that outcrossing is possible using either pigment (Larambergue, 1939) or electrophoretic (Njiokou *et al.* 1993) markers. Jarne *et al.*'s (1992) study indicates that selfing is probably high in *B. truncatus* in connection with aphally. The mating system is therefore probably a mix between selfing and outcrossing (mixed mating system) with predominant selfing. The results obtained in this study indicate that selfing must indeed occur with very high frequency since no heterozygous individual was found. Wright's fixation index is then equal to 1. Assuming the simple conditions of the mixed mating model (see Brown, 1989 for review), $S = 1$ in the population at equilibrium, since $F = S/(2-S)$ [see also equation (3) in Hedrick *et al.* 1991]. However, low levels of outcrossing would be undetectable since there is no polymorphism. Selfing only leads to a high frequency of homozygotes and cannot explain the reduced polymorphism in *B. truncatus*. Indeed numerous selfing hermaphrodite species exhibit low heterozygosity with however a variability similar to that of outcrossing species. The difference resides in the genetic variability distributed among populations rather than within populations in selfers (Allard & Kahler, 1971; Selander & Kaufman,

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Table 6. *Observed heterozygosity* (H_o) *and suggested mating system* (*C = cross-fertilization; S = self-fertilization*) *in some hermaphrodite freshwater snail species*

Species[a] (N_p)[b]	N_l[c]	H_o	Mating system	Authors
B. globosus (23)	8 (4)	0·019	C	Jelnes (1986)
B. globosus (13)	18 (9)	0·034	C	Njiokou *et al.* (in prep.)
B. umbilicatus (12)	8 (4)	0·025	C	Jelnes (1986)
B. senegalensis (3)	8 (0)	0·000	S	Jelnes (1986)
B. senegalensis (8)	15 (0)	0·000	S	Mimpfoundi & Greer (1989)
B. forskalii (22)	8 (0)	0·000	S	Jelnes (1986)
B. forskalii (32)	15 (0)	0·001	S	Mimpfoundi & Greer (1990*a*)
B. cernicus (25)	11 (7)	0·090	C	Rollinson & Wright (1984)
B. cernicus (8)	11 (7)	0·009–0·203	C	Rollinson *et al.* (1990)
B. truncatus (21)	8 (0)	0·000	S	Jelnes (1986)
B. truncatus (25)	11 (3)	0·000	S	Mimpfoundi & Greer (1990*d*)
B. truncatus (18)	29 (10)	0·000	S	This study
Bi. pfeifferi (12)	10 (7)	0·007	S	Bandoni *et al.* (1990)
Bi. pfeifferi (20)	19 (4)	0·002	S	Mimpfoundi & Greer (1990*b*)
Bi. glabrata (7)	26 (4)	0·000–0·055	C	Mulvey & Vrijenhoek (1982)
Bi. glabrata (8)	21 (13)	0·013	C	Mulvey *et al.* (1988)
Bi. camerounensis (12)	19 (5)	0·011	C	Mimpfoundi & Greer (1990*c*)
Bi. alexandrina (11)	28 (6)	0·051	C	Vrijenhoek & Graven (1992)
L. peregra (7)	12 (9)	0·218–0·266	C	Jarne & Delay (1990)
L. elodes (3)	15 (6)	0·080–0·130	C	Brown & Richardson (1988)

[a] *B.*, *Bi.* and *L.* stand respectively for *Bulinus*, *Biomphalaria* and *Lymnaea*.
[b] N_p is the number of populations studied.
[c] N_l is the number of loci studied with the number of polymorphic loci in brackets.

1973; Hamrick & Godt, 1989). The very low polymorphism within populations of *B. truncatus* could be explained by the action of both genetic drift and selfing, this latter increasing the probability of fixing an allele when compared to outcrossing.

Selfing could also be favoured by allotetraploidy (Lande & Schemske, 1985). Although the genetic polymorphism is very low in *B. truncatus*, a 'pseudo-heterozygosity' is maintained in this species, when different alleles are fixed at the two diploid homologous loci of the tetraploid locus. This is true as long as the two loci are active but not in unibanded genotypes. Thus the 'pseudo-heterozygosity' does not experience the effect of genetic drift and inbreeding compared to true heterozygosity (Wright, 1977). Whether 'pseudo-heterozygosity' could confer on tetraploid species a higher fitness than that of diploid parental species is an open question, and probably varies with local environmental conditions (Stebbins, 1977).

Brown & Richardson (1988) showed that the genetic variability in gastropods varies with the kinds of environment in which species live, increasing from the terrestrial environments to the freshwater and marine environments. Within each environment, the variability is generally higher in outcrossers than in selfers. This last tendency is illustrated for freshwater hermaphrodite snails in Table 6. We compiled all data available on natural populations. The data are unfortunately limited to three genera. The mating system has been inferred either from the analysis of population genetic structure (heterozygote deficiency and parent–offspring analysis) or from laboratory studies involving genetic markers or inbreeding depression estimation (see Jarne *et al.* 1993 for review). The low variability in *B. truncatus* observed in this study is also characteristic of other selfing species. Some of these species such as *Bulinus guernei* and *B. rohlfsi* are tetraploid (Jelnes, 1986), others such as *B. senegalensis*, *B. forskalii* and *Biomphalaria pfeifferi* are diploid. Although tetraploidy is likely to favour selfing (see above), selfing is clearly not restricted to tetraploid species. On the other hand, the genetic variability is higher in outcrossing species such as *Bulinus globosus*, and much higher in *B. cernicus* and *Lymnaea peregra* (Table 6). The reason for a difference when outcrossing species are compared is unclear. For instance, *B. cernicus* populations are highly variable although this species is restricted to Mauritius and to few populations whereas the widely distributed *B. globosus* is far less variable. In outcrossers the origin of low polymorphism may be genetic drift and founder effect (Selander & Ochman, 1983; Brown & Richardson, 1988). However, few studies have considered the role of both environmental conditions and genetic systems in snails to explain the observed polymorphism (Selander & Ochman, 1983; Mulvey *et al.* 1988; Jarne & Delay, 1991).

(ii) *Geographic variability*

Our study indicates a low genetic differentiation between populations using both genetic distances and CFA results. The population from Egypt differs from Ivory Coast populations at only two loci. Low differences are registered among Niger and Ivory Coast populations, between Niger and Ivory Coast populations, among populations from Southern and Northern Ivory Coast.

The distribution of genes among populations depends on many parameters: among others the spatial distribution of populations, the geographic distances between them, the mode and level of gene flow and the extinction/recolonization processes (Slatkin, 1985*a*; Wade & McCauley, 1988; McCauley, 1991). Slatkin (1977), Wade & McCauley (1988) and McCauley (1991) have analysed the distribution of variability using F statistics for different values of these parameters. Their results are not always intuitively obvious. For instance, the extinction rate can either increase or decrease the genetic variance among populations, depending on the model assumed. However, high gene flow clearly promotes genetic homogeneity over populations. The low differentiation of *B. truncatus* could therefore be a consequence of high gene flow between populations. In our study, the populations separated by large distances (see Fig. 1) are probably not connected by a direct gene flow, although birds are suspected of promoting migration over large distances in snails (Boag, 1986). Indirect gene flow is also possible. We also observe a positive correlation between genetic and geographic distances which points to an isolation by distance mechanism, gene flow decreasing with increasing distance. An isolation by distance mechanism could be promoted by snails sampled in different bioclimatic areas (see Fig. 1) with populations from different areas exchanging few genes (e.g. between forest and savannah). The low genetic differentiation can also be explained either by a fossil gene flow between populations, or by a colonization from a single and poorly variable population.

One has still to explain how some private alleles can be fixed. Using Slatkin's (1985*b*) method for estimating gene flow from the average frequency of private alleles, we obtained Nm of the order of 0·02–0·03 at the scale of the whole study. This value is low and could not promote the level of genetic homogeneity observed. However, the Ivory Coast populations bearing one or two private alleles are rather far away from the others and are likely to be isolated by distance. On the other hand, private alleles in Niger do not occur in remote populations. An explanation may be that once a mutation in a given population occurs, it could be driven quickly to fixation by the combined effects of genetic drift and the mating systems before being exported to other populations. It is also possible in some cases that this mutation cannot be exported because of selection, and therefore remains as a private allele.

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